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Life cycle of *Tomicus destruens* in a pine forest of central Italy

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Abstract

Tomicus destruens (Wollaston) (Coleoptera Curculionidae) is a serious threat to the stability of pine forests in the Mediterranean region and along the Atlantic coasts of Portugal, Spain and France. Despite its phytosanitary importance, some aspects of the life cycle of this bark beetle are still unclear. In this study, carried out in a pine forest of central Italy, traps baited with (-)-α-pinene were used to monitor adult flight and the wood colonized by the bark beetle was examined in order to find out more about the life cycle of the beetle and its voltinism.

At the study site, the flight of adult beetles for reproduction takes place continuously from autumn to mid-spring of the following year, with a peak of activity in the middle of autumn. Therefore, a part of the adult beetles, of the developing larvae and of the pupae overwinter under the bark of attacked pines, while a part of the adult population overwinter in the pine shoots. Adult beetles of the new generation emerge from the colonized material during the spring and estivate in the pine shoots. The bark beetles never showed flight activity in summer, nor did adults reproduce on pines at that time. Based on the data of the present study, in the environment studied *T. destruens* completes one generation per year. It remains to be seen whether some of the beetles emerging at the beginning of spring are able to start a second generation in the same year.

Key words: Bark beetles, biology, Mediterranean pine forests.

Introduction

Tomicus destruens (Wollaston) (Coleoptera Curculionidae) is a bark beetle widespread in the pine forests of the entire Mediterranean area and has also been reported from the Atlantic coastal pine forests of Portugal, Spain, France and from the island of Madeira (Chararas, 1962; Mendel *et al*., 1985; Lekander, 1971; Chakali, 1992; Monleón *et al*., 1996; Ben Jamaâ, 2000; Kohlmayr *et al*., 2002; Gallego *et al*., 2004; Faccoli *et al*., 2005b; Horn *et al*., 2006; Vasconcelos *et al.,* 2006). In Italy three *Tomicus* species occur: *T. piniperda* (L.), *T. minor* (Hartig) and *T. destruens*, but only the third occurs in coastal pine forests, as has been found by several authors working independently (Kohlmayr *et al*., 2002; Faccoli *et al.,* 2005b; Faccoli, 2006; Horn *et al*., 2006), while *T. piniperda* occurs only in the northern regions of Italy (Masutti, 1995).

Infestations of *T. destruens* have been recorded in Italy since the middle of the 20th century in stands of stone pine (*Pinus pinea* L.), maritime pine (*Pinus pinaster* Aiton) and Aleppo pine (*Pinus halepensis* Miller) (Russo, 1940; 1946; Masutti, 1969; Triggiani, 1984; Binazzi and Covassi, 1989; Nanni and Tiberi, 1997). According to Faccoli *et al*. (2005a), all Italian studies carried out in the past on *T. piniperda* in Mediterranean forests report data concerning *T. destruens.*

Adults of *T. destruens* breed under the bark of weakened trees of several *Pinus* species and cause the death of the trees within a few months (Masutti, 1969; Faccoli, 2007). The new beetles emerge over a 2-3 month period, generally during the spring or across spring and summer, depending on the geographical location. Adult beetles mature the gonads feeding in the shoots of healthy pine trees during spring and the whole summer period (Russo, 1940; Masutti, 1969; Triggiani, 1984; Santini and Prestininzi, 1991; Monleón *et al*., 1996; Nanni and Tiberi, 1997). Adult beetles overwinter in the shoots of healthy pine trees or in maternal galleries under the bark, and the juveniles overwinter in their feeding galleries (Triggiani, 1984; Monleón *et al*., 1996; Santini and Prestininzi, 1991; Faccoli *et al*., 2005a).

Despite the injuriousness of *T. destruens* in Mediterranean pine forests, some aspects of its life cycle are still unclear, and information reported in the literature sometimes disagrees. Many authors state that *T. destruens* produces only one generation per year (Dajoz, 1980; Masutti, 1969; Mendel *et al*., 1985; Chakali, 1992; Monleón *et al*., 1996; Ben Jamaâ *et al*., 2000; Faccoli *et al*., 2005a), but others report that in the coastal areas of central Italy it produces a second generation (Russo, 1940; 1946; Nanni and Tiberi, 1997; Masutti and Zangheri, 2001). It is however possible that this species develops one or more sister generations (Masutti, 1969; Mendel *et al*., 1985; Dajoz, 1980; Monleón *et al*., 1996). Moreover, in pine forests of the coasts of the northern Adriatic and in Algeria, reproductive activity of *T. destruens* takes place between the end of winter and early spring (Masutti, 1969; Chakali, 1992; Faccoli *et al*., 2005a). Ben Jamaâ *et al*. (2000) reported that colonization occurs earlier, in January, in Tunisia, whereas Mendel *et al.* (1985) found that in Israel a first phase of colonization lasted from October until December. For the coastal areas of central Italy, Nanni and Tiberi (1997) reported two generations for *T. destruens*, the first in spring, and the second between the end of summer and autumn.

The aim of the present study was to find out more about the life cycle and voltinism of *T. destruens* in a coastal pine forest of Tuscany near Marina di Alberese. At this site, there have been infestations of this bark beetle in stone and maritime pine stands for a number of years, and the aggressiveness of the beetle is related to a

general weakening of the trees that has been due to climatic difficulties and a lowering of the water table, with consequent infiltration of brackish water into the soil (Nanni and Tiberi, 1997).

Materials and methods

The study was conducted over a period of three years (2002-2004) in a coastal pine forest of Tuscany, central Italy, near Marina di Alberese (42°39'39'' N, 11°2'40'' E, 4 m a.s.l.). Most of this forest consists of *P. pinea* (20-50-year-old trees), while *P. pinaster* grows only on the littoral dunes. The mean annual temperature of the area is 14.5 °C, while in January it is 7.1 °C and in August 23.1 °C, and the mean annual rainfall is 667 mm.

Monitoring adult flight

At the end of September 2002, four funnel traps (Pherotech, Delta, B.C., CA) were placed in an area experiencing severe bark beetle infestation of stone pines. The traps were located at least 30 m apart and were baited with (-)-α-pinene (Sigma-Aldrich, St. Louis, MO, USA). They were checked every two weeks until the end of September 2003. From September 2003 to October 2004, four funnel traps, of the same type as those used in 2002, were set up in another part of the pine forest, close to the first area. These traps were checked weekly during the study period. Trapped specimens were sexed by examination of the genital apparatus.

We used (-)-α-pinene to bait the traps since this monoterpene has been effective in studies on *Tomicus* species (Byers *et al.,* 1985; Schroeder, 1998; Poland *et al*., 2003; Faccoli *et al.,* 2005a; Vasconcelos *et al*., 2005). The (-)-α-pinene dispenser was a plastic test-tube (Kartell, Milano, Italy), perforated to allow the release of the attractant (release rate 90 mg/day at + 21 °C), and was replaced every two weeks. To prevent UV degradation of the compound, Alu-paper protected plastic vials were used. The stability of the (-)-α-pinene inside the dispenser during the period in which it remained in the field was ascertained by means of GC-MS and NMR analyses. GC-MS analyses was performed with a Shimadzu GC-17A coupled with a Shimadzu GCMS-Q5050A (Shimadzu Corp., Kyoto, Japan) using a factor four capillary column FU5MS5; NMR analyses were obtained with Varian Gemini 200 and Varian Mercury 400 spectrometers (Varian Inc., Palo Alto, CA, USA). Analyses were carried out at the Organic Chemistry Institute of the University of Florence.

Weather data during the *T. destruens* flight period was provided by the Tuscan Agrometeorological Service (ARSIA). The meteorological stations were located 500 m away from the study site.

Occurrence of adult bark beetles in pine shoots during the reproductive period

Between September 2003 and April 2004, 500 pine shoots of healthy *P. pinea* trees were randomly sampled each month from trees growing in the area where the bark beetle flight was being monitored. Pine shoots were collected from the upper part of the crown using an aerial platform and a telescopic pruning wand. The shoots were checked while still in the field to detect the bark beetle in the maturation galleries.

Examination of colonized wood

Between the beginning of autumn 2002 and the end of March 2003, eight wind-felled stone pines showing signs of boring dust and/or resin tubes, an indication of bark beetle infestation, on the bark, were randomly selected in the pine forest at the time of nuptial chamber excavation or first egg laying by the female. The state of colonization of the pine by the beetle was ascertained by debarking. The date of pine tree selection (at the start of colonization) is shown in Table 1.

The colonized wood was inspected every two weeks, till the new beetles emerged, by removing the bark from two 10x20 cm rectangles per tree, in order to observe the juvenile development. In this way it was possible to follow the entire life cycle of the beetle from oviposition to the emergence of the new generation of adults, also recording the occurrence and duration of the pre-imaginal stages. In autumn 2003, in the pine forest we only found one wind-felled pine tree attacked by the bark beetle; on this tree the process of *T. destruens* colonization and development was followed until spring 2004 in the same way as it had been in 2002-2003.

In the spring of 2003 and 2004, before the emergence of the new beetles, three stone pine logs (long 1 m, Ø 25 cm), colonized by the bark beetle were placed in a rearing cage in the laboratory at room temperature in order to investigate the sex ratio. To prevent the logs from drying out, their ends were coated with paraffin. Males and females were distinguished by extracting the genital apparatus.

Identification of the species

Specimens collected in the traps and from the pine logs were examined in the laboratory and send to M. Faccoli of the University of Padua (Italy) for the correct identification of the *T. destruens* specimens.

Statistical analysis

The capture of *T. destruens* was analysed by means of ANOVA and significance by means of post-hoc Tukey test and Students *t*-test ($P < 0.05$). Data were log-transformed [x' = Log (x + 1)] to meet assumptions of normality (Zar, 1999). The female frequencies were analysed by Chi square test. All analyses were performed by SPSS program (SPSS, 1999).

Results

Adult flights

Adult *T. destruens* first became trapped at the beginning of October 2002, and flights peaked at 11/6/2002 (78.0 ± 17.6 S.E.), while there were few captures after mid-December (figure 1). Bark beetles became again more abundant in the traps in mid-March, and captures ceased definitely by the end of April 2003. No beetles were caught in summer. A mean of 167.0 (± 28.3 S.E.) beetles per trap was captured during autumn 2002-

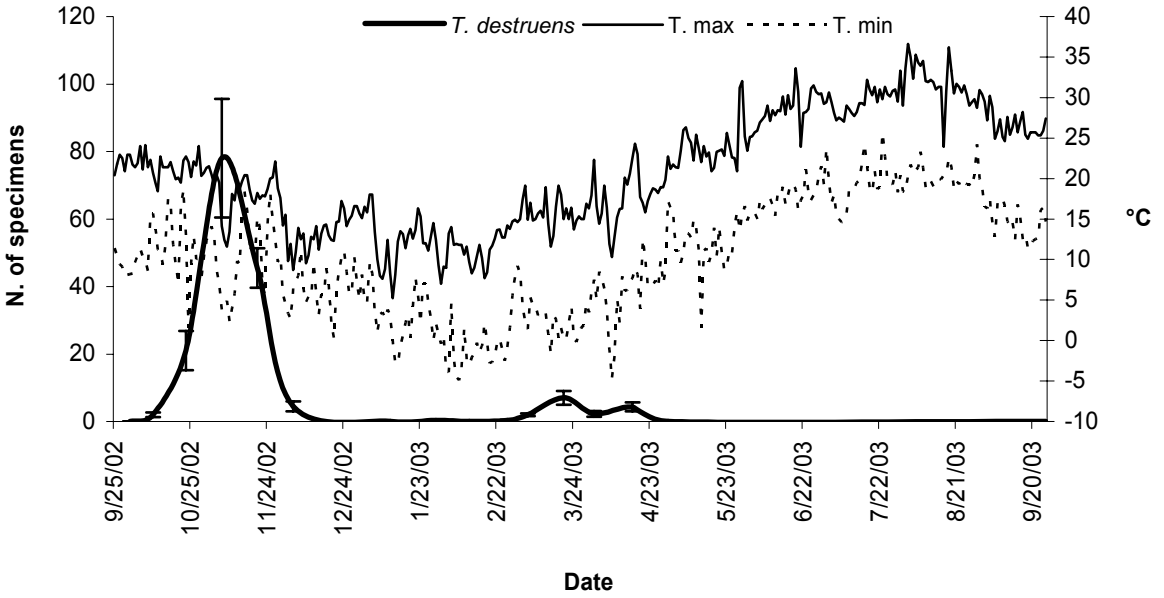

Figure 1. Mean number (±S.E.) of *T. destruens* specimens trapped at Marina di Alberese between the beginning of autumn 2002 and the end of summer 2003, and daily temperature.

spring 2003. 90.4% of all beetles were trapped in autumn, 5.7% in winter and 3.9% in spring; the number of beetles trapped in autumn was significantly higher than those trapped in winter and spring (ANOVA; $F_{2,9}$ = 23.96; P = 0.0002). In autumn, more females were captured than males, 59.6% compared with 40.4%. The percentage of trapped males decreased to 34.2% in winter and to 27.3% in spring; these differences were not significant.

In autumn 2003, adult flight began at the beginning of October (figure 2), and peaked at 11/6/2003 (21.8 ± 9.9 S.E.). Only modest numbers of adult beetles were caught in the traps from the end of December 2003 to April 2004. No beetles were caught during the remaining part of spring and the summer. Adult *T. destruens* beetles were again trapped at the beginning of October 2004. A mean of 103.3 (± 31.1 S.E.) beetles per trap was captured between autumn 2003 and spring 2004. 90.6% of beetles were captured in autumn, 7.7% in winter and 1.7% in the following spring; the number of trapped beetles was significantly higher in autumn than in winter and spring (ANOVA; $F_{2,9}$ = 9.31; P = 0.006). In autumn, males and females were captured in almost equal proportion (49.5% ♂, 50.5% ♀), but the percentage of trapped males decreased to 37.8% in winter and to 31.8% in spring; these differences were not significant.

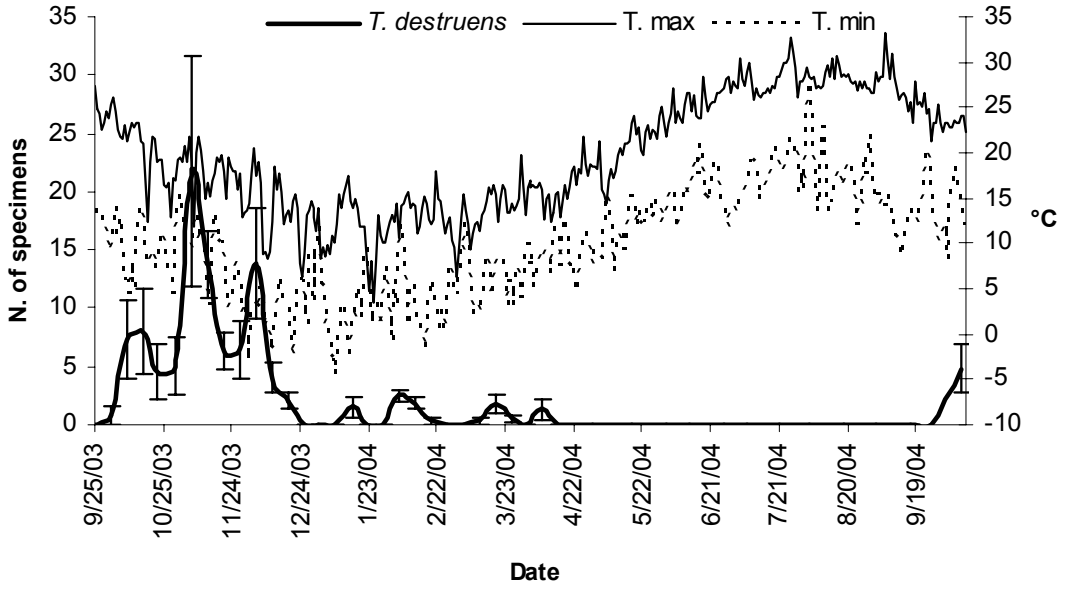

Figure 2. Mean number (±S.E.) of *T. destruens* specimens trapped at Marina di Alberese between the beginning of autumn 2003 and the beginning of October 2004, and daily temperature.

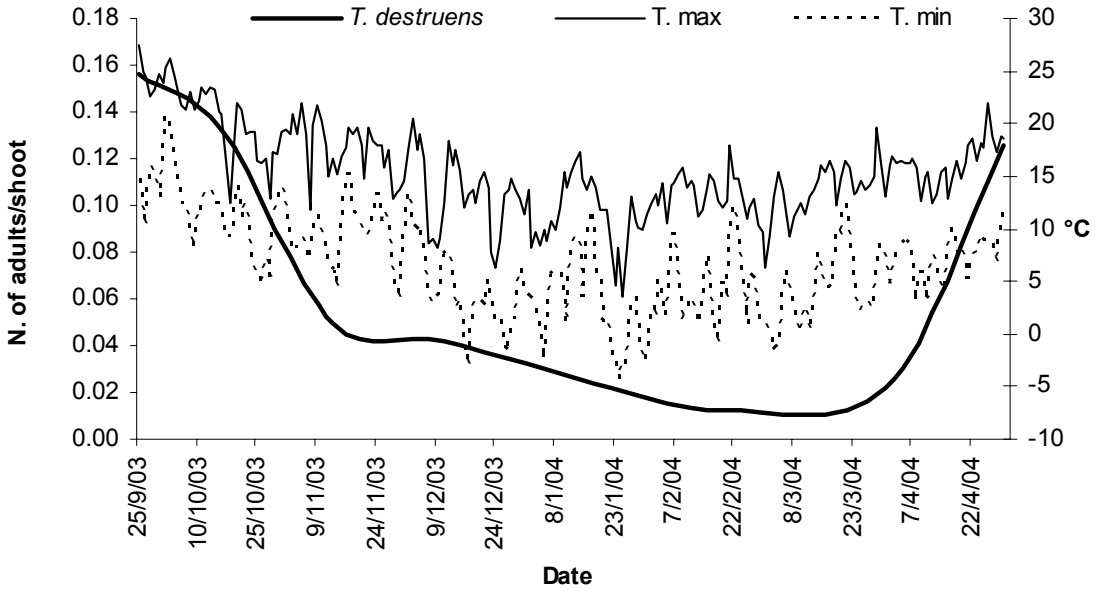

Figure 3. *T. destruens* adults on pine shoots between autumn 2003 and spring 2004, and daily temperature.

No significant differences were detected by Students *t*-test in the number of captured bark beetles in the two trapping periods (2002-2003 and 2003-2004).

The (-)-α-pinene inside the dispensers did not show any significant alterations, nor did NMR spectra show a visible sign of decomposition of the substance during the period in the field.

Presence of adult *T. destruens* in shoots during the reproductive period

Seventy-nine *T. destruens* adults were found in the 500 pine shoots checked at the end of September 2003 (figure 3). The number of adults in the shoots decreased progressively until mid-February 2004 (2/19/04), when there were only 6 specimens. The number of specimens in the pine shoots did not increase again until the end of March 2004. At the end of May 2004, the number of colonized shoots was already comparable again to the number recorded in early autumn 2003.

Development of the juvenile instars

On the 8 pine trees, *T. destruens* adults in the reproductive phase were observed under the bark from early October 2002 until the second half of March 2003. Examination of the colonized wood showed that the duration of preimaginal development varied depending on the period at which the eggs were laid. Larvae of *T. destruens* were found under the bark from October 2002 until the middle of spring 2003, while the pupae were detected from late January until May. Adults coming from larvae that had hatched at the beginning of autumn 2002 started to emerge from infested trees at the end of March 2003 (table 1). Larvae hatching at the end of winter 2003 had a shorter duration of growth, around 2-3 months, with the emergence of new adults at the beginning of June. No breeding beetles were found in the pine forest after the second half of April. Adult reproductive activity did not start again until October 2003, when we could detect it on only one wind-felled pine tree (table 1). The development of these specimens of *T. destruens* had the same duration as in the previous year.

The sex ratio of beetles emerging from infested logs reared in the laboratory in 2003 was similar to that in 2004 (42.7% ♂, 57.3% ♀ in 2003, and 43.7% ♂, 56.3% ♀ in 2004).

Table 1. *P. pinea* trees found to be attacked by *T. destruens* during the study period.

Period of colonization	No. of tree	Date of selection (at the time of beginning colonization)	Date of beetle emergence
Autumn 2002 – Spring 2003	1	6 October	28 March
	2	2 November	20 April
	3	29 November	12 May
	4	12 December	22 May
	5	4 January	22 May
	6	29 January	22 May
	7	28 February	22 May
	8	15 March	4 June
Autumn 2003 – Spring 2004	1	16 October	8 April

Discussion

Two main flight periods of *T. destruens* were found, a more intense one in autumn and a second one between the end of winter and the beginning of spring. It should be noticed however that even in winter some adult specimens were caught in the traps. This shows that the flight and reproductive activity of *T. destruens* in the environment studied never ceased completely between autumn and the following spring, as was also found by Dajoz (1980), Triggiani (1984), Mendel *et al*. (1985), Santini and Prestininzi (1991) Monleón *et al*. (1996), and Nanni and Tiberi (1997). This continuous colonization activity for such a long period (6 months) can be explained in two ways: one is by assuming that during the winter and in spring adult beetles generate 2, 3 or more sister generations after regeneration feeding in the shoots (Dajoz, 1980; Mendel and Halperin, 1982; Monleón *et al*., 1996; Ben Jamaâ *et al*., 2000; Vasconcelos *et al*., 2003); the other is that not all the beetles in the shoots start to reproduce at the same time in autumn, but that some beetles spend a longer time in the shoots, until winter or early spring of the following year (Masutti, 1969; Santini and Prestininzi, 1991). Triggiani (1984) reported that in southern Italy sister broods could appear between January and March, but Masutti (1969) stated that this phenomenon was uncommon in pine forests of the northern Adriatic region. In Portugal, Vasconcelos *et al.* (2005) supposed that the observed two main flight periods were due to the coexistence of both *T. destruens* and *T. piniperda* populations; the first species has its reproductive flight during autumn, whereas the second reproduces at the end of winter or early spring. However, this possibility can be excluded in the forest we examined since the *Tomicus* population could only be referred to *T. destruens* (Masutti, 1995; Kohlmayr *et al*., 2002; Faccoli *et al.,* 2005b; Faccoli, 2006; Horn *et al*., 2006, Faccoli, personal communication).

The flight of adult *T. destruens* was closely dependent on temperature. Ben Jamaâ *et al*. (2000) reported that in Tunisia reproductive flights resumed between February and March when the minimum daily temperatures reached 6 °C and the maximum 16 °C, while Faccoli *et al*. (2005a) examining the northern Adriatic area found that flights resumed only when the mean daily temperature reached 12 °C. In our study, the maximum temperatures during the flight periods were variable, particularly between autumn and winter-early spring. In autumn (the peak flight period), the maximum daily temperatures were 18-22 °C, and the minimum 7-14 °C; between February and March, the maximum temperatures were 10-16 °C, and the minimum ranged from -2 °C to 5 °C. In this connection, Faccoli *et al.* (2005a) postulated the existence of two *T. destruens* populations, one of which was thermophilic. This has not yet been demonstrated for *Tomicus*, but it could be true since thermophilic populations have been found for other pine xylophages, such as *Pissodes castaneus* (De Geer) (Alauzet, 1977).

The duration of preimaginal development is also highly dependent on temperature (Monleón *et al*. 1996; Faccoli *et al*., 2005a). *T. destruens* larvae that hatched in autumn required up to 6 months to reach the adult stage, while those hatching in late winter-early spring required only 2-3 months, as reported by Faccoli *et al*. (2005a). New beetles only emerged from the end of March to June, which is a short period if it is considered that reproductive activity lasts from October to April. It is also possible, at least for adults emerging at the beginning of spring that they already breed in this same season, with the emergence of a second generation of beetles in June-July. However this hypothesis of a second generation still remains to be verified and would only be possible if the gonadal maturation of *T. destruens* adults in the pine shoots took a very short time. In this connection it should be borne in mind that under laboratory conditions, *T. piniperda* can reproduce successfully after only 3 weeks of shoot feeding (Salonen, 1973) or even without any shoot feeding maturation at all, and therefore it is theoretically possible that *T. piniperda* has a multivoltine life cycle (Poland and Haack, 2000).

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